The effect of *Gratiana boliviana* (Coleoptera: Chrysomelidae) herbivory on growth and population density of tropical soda apple (*Solanum viarum*) in Florida

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RESEARCH ARTICLE

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The effect of herbivory by *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae) on the invasive, tropical soda apple (TSA) (*Solanum viarum* Dunal, Solanaceae), was investigated using exclusion methods and by monitoring the density of *G. boliviana* and the weed at four locations over a period of 40 months. TSA plants protected by insecticide were taller, wider, and had greater canopy cover that unprotected plants, and plants in closed cages were taller and wider than those in open cages. Survival of plants was higher in plots protected with insecticide than in unprotected plots in both years of a 2-year study. In the population dynamics study, the initial density of TSA was 4–5 times higher at one of the locations than at the other three sites, but within 3 years, TSA density at the high density site had declined by 90%. At the three sites which initially had a low abundance of TSA, density remained low throughout the study. The intrinsic rate of increase of *G. boliviana* varied between –3.9 and 4.5, but over the 3-year study, was not different from zero, indicating a stable population. The intrinsic rate of increase was lower than zero for the period from October to January, and greater than zero during the January to April period. In the periods from April to July and July to October, the rate of increase was not different from zero. The implications of these results for biological control of TSA in Florida are discussed.

Keywords: weed; biological control; evaluation; intrinsic rate of increase; exclusion

Introduction

Tropical soda apple (TSA), *Solanum viarum* Dunal (Solanaceae), is a prickly, perennial weed from South America that was first reported in Florida in 1988 (Mullahey, Nee, Wunderlin, and Delaney 1993). It invaded rangelands, improved pastures and natural areas (Mullahey 1996), and rapidly spread throughout Florida and into other southern states (National Plant Data Center 2010). Cattle and wild mammals (e.g., deer, feral pigs) do not consume the prickly leaf tissue, but they do feed on the fruits, transporting seeds in their digestive tracts to new areas (Brown, Mullahey, and Akanda 1996). Seeds also are transferred to new areas through the movement of contaminated hay and sod (Mullahey, Shilling, Mislevy, and Akanda

1998). Economic losses attributed to TSA include lower stocking rates in TSA infested pastures, and heat stress of livestock due to restricted access to shade in wooded areas dominated by TSA (Mullahey et al. 1998). A survey conducted in 2006 by Thomas (2007) indicated that Florida ranchers annually spent an average of $62/ha on chemical control and $47/ha on mechanical control of TSA. Statewide, this amounted to $6.5–16 million in estimated annual control costs. TSA also is a host of numerous plant pathogens, and may serve as a reservoir for pathogens that infect solanaceous crops, such as tomatoes, peppers, eggplants and potatoes (McGovern, Polston, and Mullahey 1994, 1996; Adkins, Kamenova, Rosskopf, and Lewandowski 2007).

A leaf feeding beetle from South America, *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae), was introduced into Florida as a biological control of TSA and first released in 2003 (Medal et al. 2007). By October 2008, a consortium of state and federal agencies had reared nearly 180,000 beetles and released them at over 340 locations (Overholt et al. 2009). A survey conducted in the fall of 2008 revealed that the beetles were present at >70% of randomly selected TSA infestations in central peninsular Florida (26–29° latitude) and that plant performance, measured by height, canopy diameter, cover and number of fruit, decreased as the number of beetles and beetle damage increased. Beetles were absent from locations above 29° latitude (Overholt et al. 2009).

Gratiana boliviana adults and larvae consume foliage of TSA, and cause a distinctive shot-hole feeding pattern on the leaves. Eggs are laid individually on the upper or lower surfaces of leaves, and eclose after about 5 days at 25.5°C. Larvae complete five instars in 15–18 days, and then pupate on the underside of leaves. A generation is completed in approximately 1 month (Diaz et al. 2008). Adult beetles enter diapause during the winter months from about November to March in central Florida, and during this period do not reproduce and feed only sporadically on warm days (R. Diaz, unpublished).

The objectives of the present study were to measure the effect of *G. boliviana* defoliation on the performance of TSA in the field, and to examine the seasonal dynamics of both TSA and the beetles.

Materials and methods

Insects

A colony of *G. boliviana* was initiated with individuals received from a colony maintained at the University of Florida in Gainesville, which in turn, originated from insects collected in southeastern Brazil in the early 2000s. Insects were maintained outdoors in screen cages ($2.5 \times 2.5 \times 3$ m) on TSA plants grown from field collected seeds. Prior to planting, seeds were treated with a saturated solution of trisodium phosphate (TSP) to remove surface contamination by tobamoviruses (Adkins et al. 2007). Seeds were first sown in flats in Fafard® Superfine Germinating Mix, and then transplanted to nursery pots (16×19 cm) containing Fafard® 3B potting soil at the 5–7 true leaf stage (ca. 10 cm). At the time of transplanting, each pot received 4 g of Osmocote® slow release fertilizer (19-6-12, NPK, Scotts-Miracle-Gro®, Marysville, OH, USA). Plants were placed in cages after attaining a height of 30–50 cm, and were replaced when approximately 50% of the foliage was consumed.

During winter months (November–March), the colony was maintained in a laboratory on TSA plants in cages (60 × 60 × 60 cm, BugDorm® 2; Bioquip, Rancho Dominguez, CA, USA) at a photoperiod of 14 h L:10 h D to preclude initiation of reproductive diapause.

Effect of imidacloprid on TSA

A preliminary study was conducted to determine whether the insecticide imidacloprid had any effect on growth or fruit production of TSA. A group of 16 one-month-old TSA plants (20–30 cm ht) in 3.82-L plastic pots were randomly divided into two groups of eight plants. One group received one application of 1 mL of Admire® 2F in 207 mL of water, while the other group received 207 mL of water only. Plants were maintained for 2 months in a greenhouse, and watered as needed. Plants in both treatments also received an initial application of 4 g of Osmocote® fertilizer. Plant height, canopy diameter and numbers of flowers and fruits were measured weekly.

Exclusion study

This 2-year study was conducted on a privately owned ranch in Saint Lucie Co., Florida, from March to October in 2008 and 2009. The vegetation on the ranch consisted of a mosaic of open pasture dominated by bahiagrass (*Paspalum notatum* Flugge), interspersed with wooded areas (locally, and hereafter referred to as 'hammocks') dominated in the overstory by oak (*Quercus* spp.) and cabbage palm (*Sabal palmetto* (Walter) Lodd. ex. Schult. and Schult.). TSA occurred on the ranch, but its density was suppressed by *G. boliviana*, which had been released in August 2004 and become well established (Overholt, unpublished). In March 2008, a plot of TSA was established in a partially shaded hammock area of the ranch. TSA seedlings (∼10 cm tall) were planted in 12 rows of 12 plants, with 122 cm between rows and between plants within rows. Plants were irrigated regularly for 1 month after planting to improve establishment. The field was divided into 16 plots of 9 plants (3 rows × 3 plants/row), and 8 of the plots were randomly selected to receive application of imidacloprid (Admire® 2F), whereas plants in the other 8 plots received 207 mL of water. The insecticide was applied 3 weeks after planting as a drench at the base of each protected plant at a rate of 1 mL Admire® 2F in 207 mL of water. Insecticide or water was reapplied using the same rate every 2 months until October when the study ended.

In 2009, the exclusion study was conducted a second time, but modified to include four treatments; unprotected, imidacloprid protected, closed cage and open cage. The cages were constructed from mosquito nets (Nicamaka.com, Miami, FL) placed over PVC frames (1.2 × 1.6 × 2.0 m, H × W × L). To seal the closed cages, the netting at the bottom was covered with soil. The netting of the open cages was rolled up such that the bottom 40–50 cm was open. Four plots of 4 plants were randomly selected and treated with imidacloprid and 4 plots of 4 plants received only water (unprotected). Closed cages (4) were placed over 8 plants (2 per cage) and open cages (4) were placed over 8 plants. The initial height of TSA at planting was 20–30 cm. The rate of imidacloprid applied to plants was the same as used in 2008, and the first treatment occurred on March 30, 3 weeks after planting. Follow up treatments with imidacloprid, and application of water to plants in untreated plots, were made in

June and September. Plants were inspected once a month from April to October. On each sampling occasion, the following parameters were measured or estimated:

Plant height:	Height of plant at highest green foliage.
Plant diameter:	Diameter of the canopy at the widest point.
Cover:	A visual estimation of the percent of ground surface covered by the plant canopy.
	Cover was scored on a scale of 1–5 as follows; 1 = 0–19% cover, 2 = 20–39%, 3 = 40 = 59%, 4 = 60–79% and 5 = 80–100%.
Number of fruit:	Number of fruit per plant (number of fruit could not be compared between plants in closed cages with other treatments as the cages excluded pollinators, and thus no fruits were produced on plants inside cages).
Number of *G. boliviana*:	Number of *G. boliviana* larvae, pupae and adults per plant.
G. boliviana damage:	A visual estimation of the percentage of leaf area consumed by *G. boliviana* on a scale of 0–5 with $0 \leq 1\%$ leaf tissue missing, $1 \geq 1$–19%, 2 = 20–39%, 3 = 40–59%, 4 = 60–79% and $5 \geq 80\%$.
Damage by other foliage feeders:	Foliar chewing damage to plants which differed from characteristic *G. boliviana* damage (e.g., holes in leaves larger than those typically caused by *G. boliviana* and/or feeding from the leaf margin) was noted as present or absent. When non-*G. boliviana* folivores were found on plants during sampling, they were identified as either 'lepidoptera larva' or 'Colorado potato beetle' (*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)) as no other folivores were encountered during the study.
Presence of disease:	The presence/absence of symptoms of plant pathogen infection was noted in 2008. In 2009, disease symptoms were divided into three categories; *Alternaria* spp. + *Phomopsis* spp., powdery mildew, and viral infection.

Population dynamics study

This study was conducted on a second privately owned ranch in St. Lucie County, Florida. The vegetation on the ranch was similar to that described for the exclusion study. A preliminary survey conducted in early June 2006 found no *G. boliviana*, or plants which exhibited characteristic symptoms of *G. boliviana* feeding, on the ranch. TSA was found to be almost entirely restricted to hammock areas due to TSA control by mowing in open pastures. Thus, four hammock areas along a northwest to southeast axis were selected as study sites (Figure 1). On 7 June 2006, 800 adult *G. boliviana* were liberated in Release site 1 and 350 in Release site 2, with the number of beetles released approximately proportional to the area of each hammock. Beetles were released by placing 10–20 individuals on haphazardly selected plants spread throughout the hammock. Two other hammock areas (Control sites 1 and 2) were selected as controls, with the closest control being 1640 m from the nearest release site, and the second control site 764 m further away. The geographic coordinates of the borders of each hammock were recorded using a hand-held GPS and uploaded to ArcMap 9.2 (ESRI, Redlands, CA). A polygon connecting the border coordinates
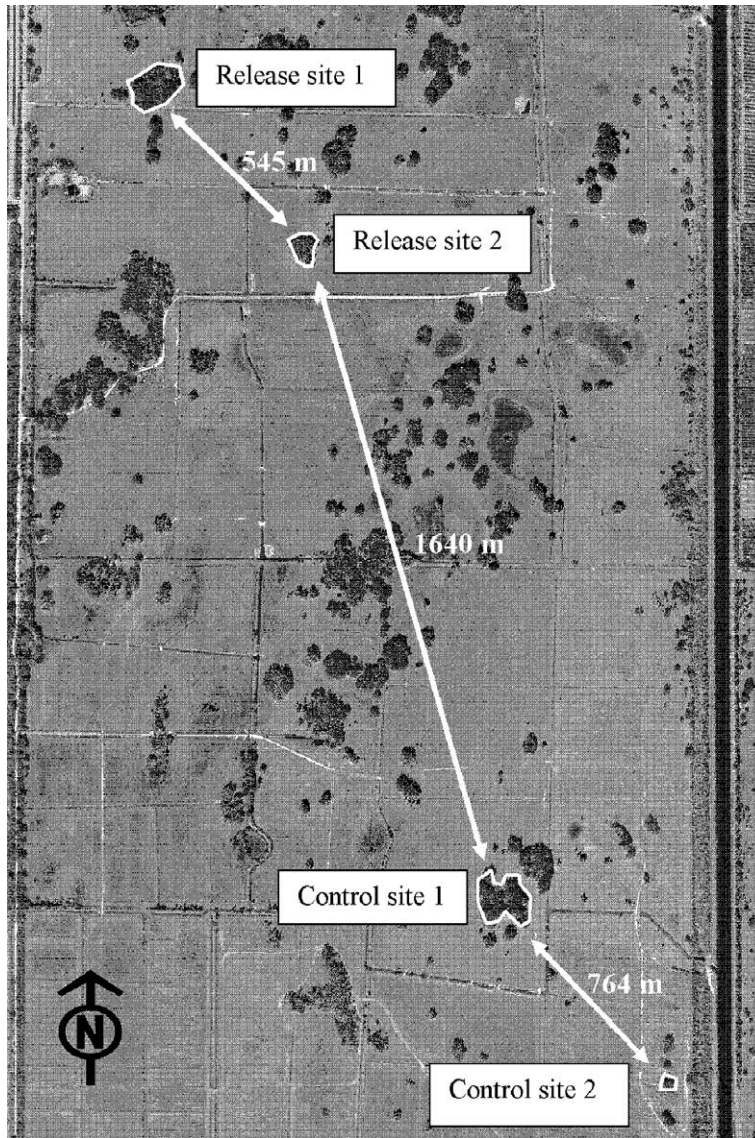
Figure 1. Aerial photograph of the population dynamics study area in western Saint Lucie Co., Florida.

was drawn around each hammock and the area determined using the MEASURE function. The sizes of the hammocks were as follows; Release site 1 = 8317 m^2, Release site 2 = 2652 m^2, Control site 1 = 9192 m^2 and Control site 2 = 1593 m^2.

The hammock areas were sampled every 3 months from 7 July, 2006 to 10 October, 2009, for a total of 14 sampling occasions. A transect dissecting each hammock through the center from east to west was established. Four perpendicular transects were randomly placed along the main transect, and two 4-m^2 (2 × 2-m) quadrates were randomly placed on the north and south sides of each of the four

perpendicular transects, such that there were 16 sampling quadrates in each hammock area. The quadrates were permanently marked with flags and sampled throughout the study. On each sampling occasion, the following parameters were estimated:

TSA plants/quadrate:	The number of TSA plants in each quadrate.
TSA plants with fruit:	The number of plants with fruits in a quadrate.
TSA cover:	The percentage of each quadrate covered with TSA visually estimated using the Braun-Blanquet (1964) method with 0 = no plants, 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, 5 ≥ 75% cover.
Number of *G. boliviana*:	The number of *G. boliviana* (larvae + pupae + adults) on the TSA plant closest to the center of the quadrate.
Gratiana boliviana damage:	The percentage of leaf area consumed by *G. boliviana* was visually estimated using a scale of 0–5 with 0 ≤ 1% leaf tissue missing, 1 ≥ 1–19%, 2 = 20–39%, 3 = 40–59%, 4 = 60–79% and 5 ≥ 80%.

Data analysis

For the preliminary study on the effect of imidacloprid on TSA, plant height, diameter and number of fruits + flowers were compared at 8 weeks after application between protected and unprotected plants with analysis of variance (PROC GLM, SAS Institute 2001). For the exclusion study, plant height, diameter, cover and number of fruit were compared between protected and unprotected plants with repeated measures analysis of variance (PROC GLM, SAS Institute 2001). Because the data from the 2009 study suggested that cages had a positive effect on plant performance, single degree of freedom contrasts were used to compare insecticide treated plants with unprotected plants and to compare the performance of plants in closed cages with those growing in open cages. The proportion of plants which survived in insecticide protected and unprotected TSA plots were arcsin square root transformed and compared with repeated measures analysis of variance. The proportion of plants which had foliar chewing damage not due to *G. boliviana* was compared on each sampling date between insecticide protected and unprotected plants, and between cages and uncaged plants, with a *G*-test of independence (Sokal and Rohlf 1995). The proportions of plants exhibiting disease symptoms in 2008 were arcsin square root transformed and compared between treatments with repeated measures analysis of variance (PROC GLM). In 2009, nearly all plants in all treatments were infected with *Alternaria* spp. or *Phomopsis* spp. by the second sampling date, and therefore no statistical comparison was made.

In the population dynamics study, TSA densities were compared between the four Octobers included in the study within each study site, and between study sites within Octobers, with one-way analysis of variance. The intrinsic rate of population increase of *G. boliviana* was calculated for each sampling occasion and study site as $r = \ln(R_o)/t$ where R_o is the net reproductive rate and t is time in months. For all time periods from October 2006 to the end of the study, R_o was calculated as the cubic root of N_{t+3}/N_t, where N_t was the population size estimated from sampling data at time t, and N_{t+3} was the population estimate at time $t+3$ months. In order to allow

calculations for sampling dates when no beetles were found, 1×10^{-6} was added to all beetle density estimates. R_o for the period of June to July 2006 was the number estimated from the July sample divided by the number released in Release sites 1 and 2. R_o was not calculated for June–July 2006 for the two control sites as beetles did not appear at those sites until October 2006. Population size estimates of *G. boliviana* were calculated for the complete area of each hammock, and derived by converting the mean number found per 4 m^2 to the expected number in the total area of each hammock. The mean intrinsic rate of increase was calculated for each 3-month period between July 2006 and October 2009 using each hammock as a replicate, and the means tested for difference from zero using two-tailed *t*-tests. The intrinsic rate of increase was also calculated for each of the four sampling periods (January–April, April–July, July–October and October–January) and tested for difference from zero with a two-tailed *t*-test.

Results

Effect of imidacloprid on TSA

The preliminary experiment conducted to determine whether there were any positive or negative effects of imidacloprid on TSA performance showed that after 8 weeks, height, diameter and the combined number of flowers and fruits did not differ between imidacloprid treated and untreated TSA plants ($F_{1,14} = 0.34$, $P = 0.57$; $F_{1,14} = 2.55$, $P = 0.13$; $F_{1,14} = 0.55$, $P = 0.47$ for height, diameter and combined number of flowers and fruits, respectively).

Exclusion study

In the 2008 field study, TSA plants protected by insecticide were taller, wider, and had greater canopy cover than unprotected plants (repeated measures ANOVA; height: $F_{1,28} = 5.21$, $P < 0.03$, diameter: $F_{1,28} = 5.75$, $P < 0.02$, cover: $F_{1,28} = 5.61$, $P < 0.03$) (Figure 2). The number of fruits produced by protected and unprotected plants was not different ($F_{1,28} = 2.24$, $P > 0.14$). In 2009, plants protected in cages outperformed plants in the other treatments (repeated measures ANOVA height: $F_{3,44} = 22.99$, $P < 0.0001$, diameter: $F_{3,44} = 19.77$, $P < 0.0001$, cover $F_{3,44} = 6.15$, $P < 0.0004$). Insecticide protected plants were taller, wider and had more fruits than unprotected plants (single degree of freedom contrasts; height: $F_{1,30} = 9.88$, $P = 0.004$; diameter: $F_{1,30} = 14.31$; fruits: $F_{1,30} = 7.47$, $P = 0.01$), but cover was not different ($F_{1,30} = 3.52$, $P = 0.07$) (Figure 3). Plants in closed cages outperformed plants in open cages with height and diameter being significantly greater in the closed cages (height $F_{1,14} = 5.28$, $P = 0.04$, diameter $F_{1,14} = 4.81$, $P = 0.05$), but cover was not different ($F_{1,14} = 2.63$, $P = 0.13$) (Figure 4). Survival of plants was higher in plots protected with insecticide than in unprotected plots in 2008 ($F_{1,14} = 6.59$, $P = 0.022$) and in 2009 ($F_{1,6} = 10.29$, $P = 0.0075$) (Figure 5).

In 2008, the proportion of plants with foliar chewing damage due to insects other than *G. boliviana* ranged between 0 and 19% for insecticide protected plants and 0–31% for unprotected plants in the first 4 months of the study (April–July). Non-*G. boliviana* damage was not observed during the last 3 months. The proportion of plants exhibiting non-*G. boliviana* damage was not different between treatments on
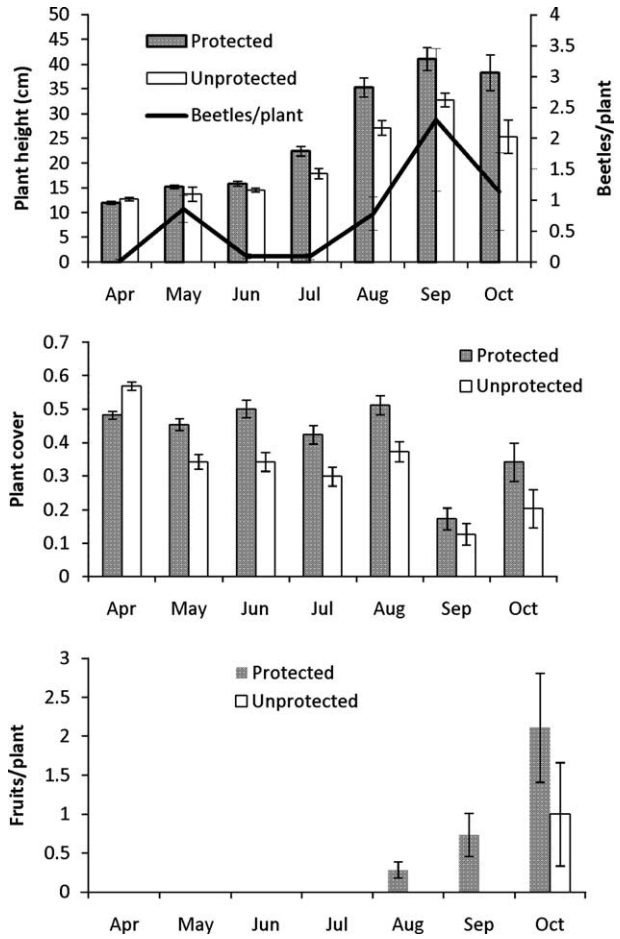
Figure 2.   Performance of insecticide protected and unprotected tropical soda apple plants on a ranch in Saint Lucie, Co., Florida from April to October 2008. Beetle numbers are counts of *G. boliviana* on unprotected plants.

any sampling date ($G = 1.03$, 0.60, 1.36, 1.92 for April, May, June and July, respectively, $P < 0.05$). In 2009, damage due to non-*G. boliviana* feeding was only observed on the first two sampling dates and restricted to non-caged plants. Non-*G. boliviana* damage was not different between protected and unprotected plants ($G = 0.80$ and 0.23 for weeks 1 and 2, respectively, $P < 0.05$). The decline in plant height and cover after June 2009 was due to heavy rainfall from June 29 to July 3 which caused flooding of the field plot. Flooding is known to stress TSA (Mullahey et al. 1998).

In 2008, there was no difference in the proportion of plants expressing symptoms of disease between protected and unprotected plants ($F_{1,11} = 2.71$, $P = 0.13$) and by June, nearly 100% of plants had disease symptoms. In 2009, all plants expressed symptoms of fungal infection by *Alternaria* spp. or *Phomopsis* spp. by May, and the incidence remained near 100% until the end of the study in October. Powdery mildew was only seen on plants in open and closed cages in May (5 out of 8 plants in open
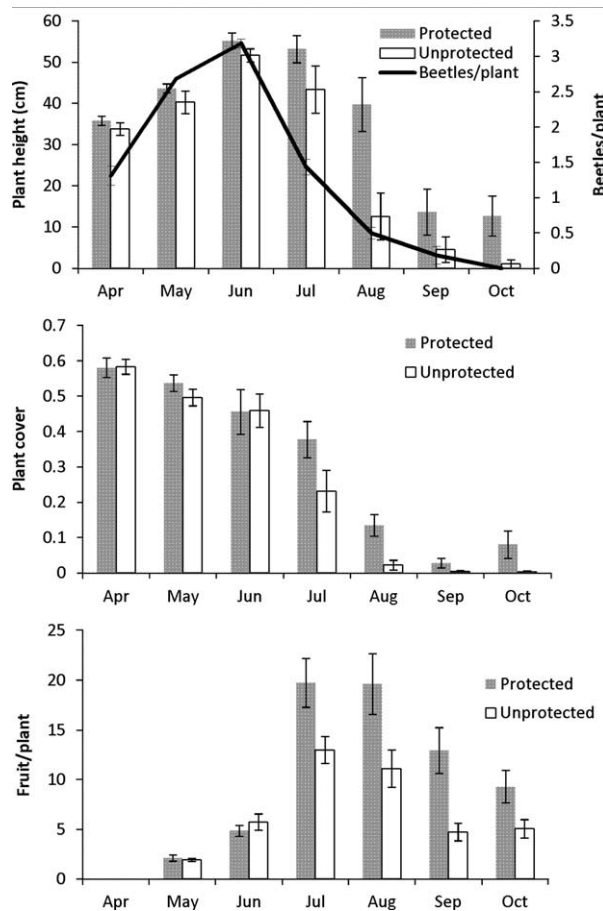
Figure 3. Performance of insecticide protected and unprotected tropical soda apple plants on a ranch in Saint Lucie, Co., Florida from April to October 2009. Beetle numbers are counts of *G. boliviana* on unprotected plants.

cages and 3 out of 8 in closed cages) and in June (2 out of 8 plants in open cages). No plants with viral symptoms were observed during the study, possibly due to treatment of seeds with TSP prior to planting.

Population dynamics study

The temporal changes in TSA and *G. boliviana* density at the four study sites are shown in Figure 6. The two control sites were quickly compromised as beetles were found at both sites during the second sampling in October 2006, indicating that the beetles had moved at least 1640 m in 4 months. Release site 1 had a much higher density of TSA (10.19 ± 1.67 plants/4 m^2, mean $\pm$ SE) at the beginning of the study compared to the other 3 sites (2.06 ± 0.45, 2.68 ± 1.67 and 2.56 ± 0.56 for Release site 2, Control site 1 and Control site 2, respectively; $F_{3,63} = 12.21$, $P < 0.0001$), but was reduced to approximately the same density as the other sites within two years. TSA
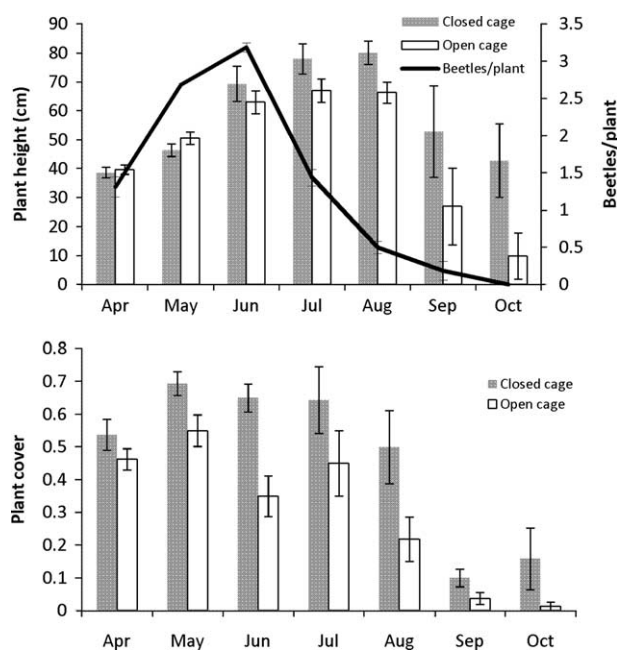
Figure 4. Performance of tropical soda apple plants in closed and open cages on a ranch in St. Lucie Co., Florida April to October 2009. Beetle numbers are counts of *G. boliviana* on unprotected plants.

density at Release site 2 decreased from October 2006 to October 2007, but thereafter remained stable (Table 1). Density of TSA at the two control sites was not different between the four Octobers included in the study. At the end of the study in October 2009, there was no difference in TSA density at the four sites (Table 1).

The intrinsic rate of increase of *G. boliviana* varied between –3.9 and 4.5 (Figure 7), but over the 40-month study was not different from zero ($t = -0.0028$, $P = 0.998$). The rate of increase was significantly greater than zero in October 2006, April 2008 and April 2009, and significantly less than zero in January 2007 and October 2008 (Figure 7). Comparing the rate of increase during the four sampling periods, it was significantly lower than zero for the October–January period and significantly greater than zero in the January to April period. In the April–July and the July–October periods, the rate of increase was not different from zero (Table 2).

Discussion

Evaluation of the impact of introduced natural enemies on target weed populations is a critical, but often neglected, component of classical weed biological control programs (McFadyen 1998; Myers and Bazely 2003; Morin et al. 2009). Impact assessment is required in order to determine whether a desired level of success has been achieved, or whether additional agents are needed to further suppress a target weed. Additionally, future support for classical biological control is contingent upon evidence of its effectiveness in regulating target weeds (McClay 1995; McFadyen 1998). The current study examined the impact of the leaf feeding beetle, *G. boliviana*,
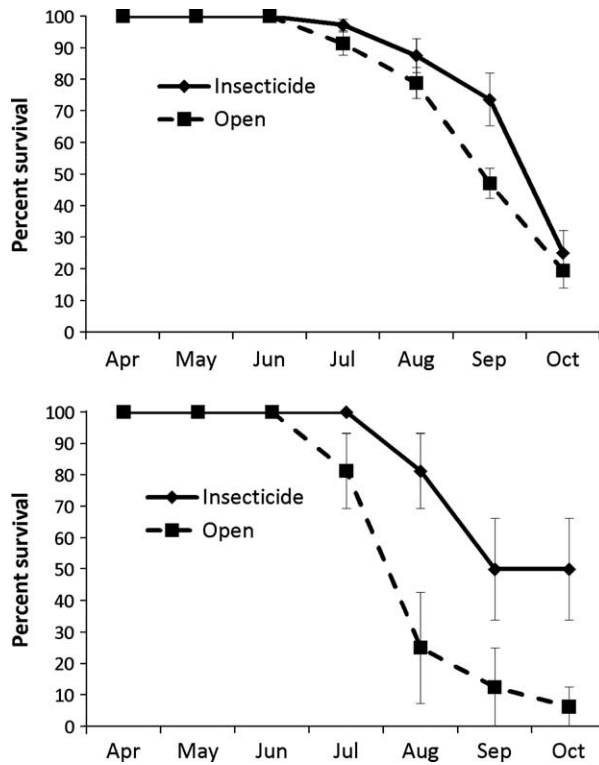
Figure 5.   Proportion of insecticide protected and unprotected TSA plants surviving in 2008 (left) and 2009 (right) on a ranch in St. Lucie Co., Florida.

on the invasive pasture weed, tropical soda apple. The exclusion study demonstrated that insect herbivory had a negative effect on individual TSA plant performance and survival. *Gratiana boliviana* was the major insect herbivore of TSA during the study, and therefore we contend that it was primarily responsible for the inferior performance of unprotected plants compared to protected plants. The most frequently observed herbivores of TSA other than *G. boliviana* in our field plots were lepidopteran larvae, but their density was low and their presence sporadic. Colorado potato beetles, which are known to occasionally reach damaging levels on TSA (McGovern et al. 1994), were only seen in very low numbers in 2008 (3 unprotected plants on one sampling occasion), and not at all in 2009. Damage due to non-*G. boliviana* folivores was not different between treatments, which is not surprising as imidacloprid is mainly active against sucking insects and beetles, and it is not labeled for control of lepidoptera (Palumbo 2001; Bayer CropScience 2003). Thus, we believe that the superior performance of protected plants was due largely to the absence of *G. boliviana*.

Cages, both open and closed, had a positive effect on plant performance. The cage environment may have conserved soil moisture, increased humidity and/or increased temperature (Van Driesche and Bellows 1996; Morin et al. 2009). Powdery mildew was only observed in cages, which may have been due to a more humid environment caused by reduced air flow. It is possible that the positive effect of the
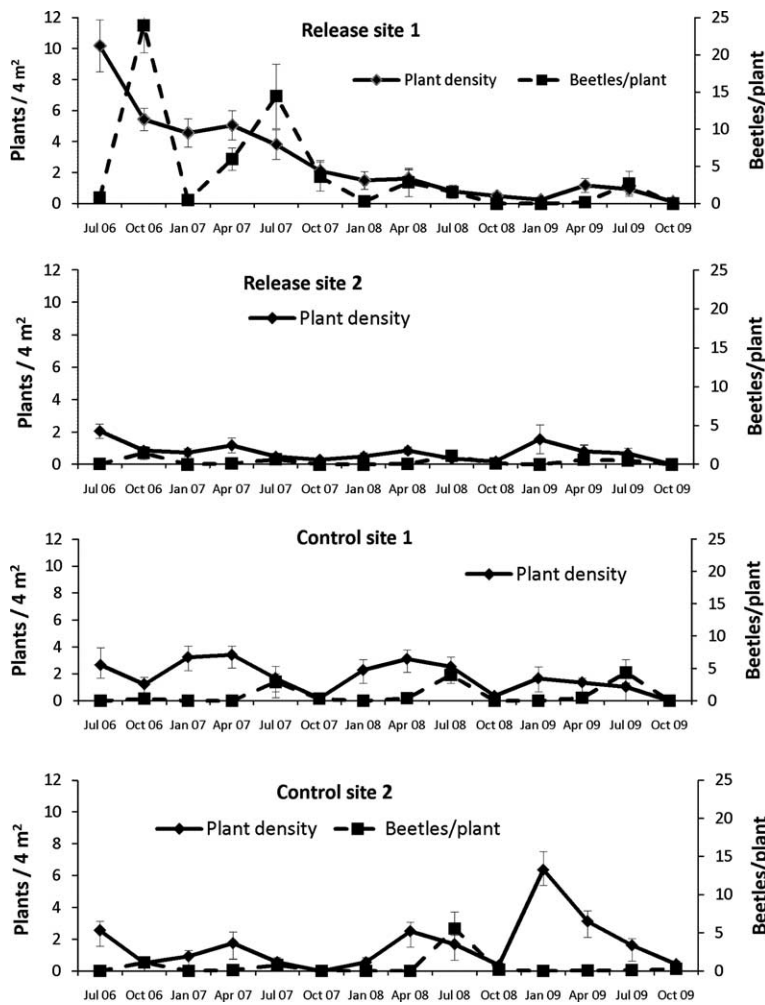
Figure 6.   Density (mean ± SE) of tropical soda apple and *Gratiana boliviana* at four locations from July 2006 to October 2009.

cage environment on plant growth was stronger in closed cages than in open cages, and therefore may have partially confounded the effect of excluding insect herbivores. However, based on the consistent differences between the insecticide protected and unprotected plants in the open, the difference in plant performance between open and closed cages is likely to be due mostly to differences in herbivory. The results of this study support earlier findings of a statewide survey in Florida which found that the presence of *G. boliviana* was associated with smaller TSA plants and fewer fruit (Overholt et al. 2009).

Evaluation of biological control programs most often focuses on the effects of agents on individual plant performance (Thomas and Reid 2007), as was the case in our exclusion study. However, reduced plant performance may have little effect on plant density (Crawley 1989), which is the preferred metric for assessing impact (Myers and Bazely 2003). The population dynamics study measured the effect of

Table 1. Density (mean plants/4 $m^2 \pm$ SE) of tropical soda apple plants in October at four study sites.

	Density (mean $\pm$ SE)[1]					
Month/year	Release site 1	Release site 2	Control site 1	Control site 2	$F_{3,63}$	P
Oct 2006	5.44 $\pm$ 0.72Aa	2.13 $\pm$ 0.65Ab	0.50 $\pm$ 0.22Ac	0.12 $\pm$ 0.09Ac	23.51	<0.0001
Oct 2007	0.88 $\pm$ 0.18Ba	0.31 $\pm$ 0.15Bb	0.19 $\pm$ 0.10Ab	0.00 $\pm$ 0.00Ab	8.72	<0.0001
Oct 2008	1.25 $\pm$ 0.51Ba	0.19 $\pm$ 0.10Bb	0.38 $\pm$ 0.15Ab	0.13 $\pm$ 0.09Ab	3.58	0.019
Oct 2009	0.50 $\pm$ 0.24Ba	0.00 $\pm$ 0.00Ba	0.38 $\pm$ 0.15Aa	0.44 $\pm$ 0.20Aa	1.63	0.1915
$F_{3,63}$	24.37	8.54	0.61	2.49		
P	<0.0001	<0.0001	=0.61	=0.07		

[1]Means followed by the same upper case letter in the same column or followed by the same lower case letter in the same row are not statistically different ($P > 0.05$).

G. boliviana on plant density over a moderately long period of 40 months. Originally, we designed the study to include two release sites and two control sites, with the expectation that the control sites would remain free of *G. boliviana* for several sampling seasons to allow comparison of TSA performance at sites with and without beetles. However, beetles moved more quickly than anticipated, and had invaded the two control sites within 3 months. During the summer of 2006, beetles were released not only at the study site, but also at several nearby locations, the closest being 3.62 km from Control site 2. It is possible that the beetles which invaded the control sites did not originate exclusively from Release sites 1 or 2, but also from another nearby release. Overholt et al. (2009) estimated that on average beetles moved 4.7 km/year. In this study, beetles moved at least 1.64 km in 4 months, and if we assume that all long distance movement occurs during an 8-month period between March and October when beetles are active, then the annual distance beetles could have moved was at least 3.28 km, which agrees fairly closely with the 4.7 km/year estimate.

The dynamics of TSA populations differed between the four sites. At Release site 1, which had a much higher density of TSA at the beginning of the study than the
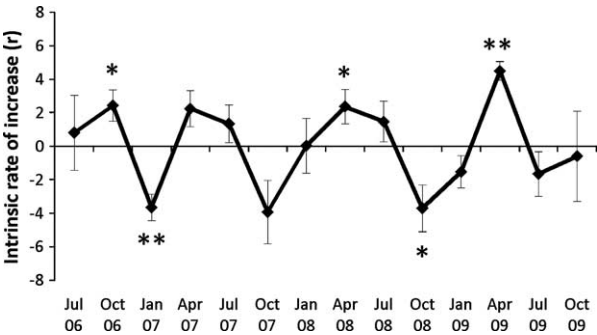

Figure 7. Intrinsic rate of increase (mean $\pm$ SE) of *Gratiana boliviana* from July 2006 to October, 2009 on a ranch in Saint Lucie Co., Florida. One asterisk indicates that a value is statistically different from zero at $P < 0.10$. Two asterisks indicate statistical different from zero at $P < 0.05$.

Table 2. Intrinsic rate of increase of *Gratiana boliviana* during four periods of the year.

Period	Intrinsic rate of increase ($\pm$SE)	t[1]	Probability
October–January	-1.70 ± 0.77	-2.21	0.049
January–April	3.06 ± 0.57	5.37	0.0002
April–July	0.46 ± 0.70	0.66	0.520
July–October	-1.43 ± 1.06	-1.35	0.198

[1]t-Statistic tests difference from zero (two-tailed).

other three sites, the TSA population declined by approximately 90%, with most of the decrease occurring during the first 2 years. At the other three sites, the TSA density was initially low, and remained more or less stable during 3 years. These results suggest that, at least under the conditions encountered during this study, *G. boliviana* was able to regulate TSA at an average density of approximately 1 plant/4 m², and that the beetles were able to reduce TSA to this density in about 2 years. One data point which did not fit this generalization was the high number of TSA plants found in Control site 2 in January 2009. Among the four sites, Control site 2 was the most highly disturbed, and appeared to be a favored resting location for cattle; on several occasions cattle had to be chased out of this hammock to allow sampling. Cattle do not consume TSA foliage, but they readily consume fruits, and are a primary means of seed dispersal (Brown et al. 1996). The high number of TSA plants found in January 2009 in Control site 2 is likely the result of defecation of a large number of TSA seeds by cattle in previous months, and soil disturbance which enhanced germination and seedling establishment. The TSA population in Control site 2 declined by April, and further by July, which we believe was due to a numerical response of *G. boliviana* to an increase in food resource availability. *Gratiana boliviana* has a generation time of approximately 1 month (Diaz et al. 2008), so our quarterly sampling could easily have missed a resurgence of beetles occurring in May or June.

TSA is an indeterminate, perennial plant which continues to grow as long as temperatures remain above freezing. When temperatures drop below freezing, above-ground parts of the plants wilt and die, but the roots remain alive and new shoots appear with a few weeks (Mullahey et al. 1998). During the course of this study, there were only a few very light freezes, and TSA populations were not heavily impacted. TSA density tended to be lowest in October, increased during the winter, and then declined during summer months. We hypothesize that this seasonal dynamic is due to a release of plants from herbivore pressure in the winter when *G. boliviana* is in diapause from about November to March. *Gratiana boliviana* population density was typically highest in July, which follows a period of positive population increase from January to July (Figure 7). The positive population growth of *G. boliviana* in April is somewhat surprising because diapause does not terminate until about the middle of March (Diaz, unpublished data), allowing only 3–4 weeks for the beetle population to increase. Thus, the positive population growth between January and April only occurs during the later weeks of the period, and reflects population increase over one generation.

Hoffman (1995) described success in weed biological control programs as being 'complete' when no other control tactics are required, 'substantial' when density is

reduced but other tactics are occasionally required and 'negligible' when control still depends largely on tactics other than biological control. A survey conducted in 2006 indicated that the majority of ranchers in central Florida considered TSA to be the most serious pasture weed problem, and that 75% of ranchers controlled it using chemical and/or mechanical means (Saluadeen 2006). Using Hoffman's definition, success of biological control of TSA in 2006 would have been categorized as negligible. However, *G. boliviana* was not released in Florida until 2003, and probably did not become widely present in south/central Florida until 2008 when the number of beetles released increased dramatically (Overholt et al. 2009). In the fall of 2008, a survey of randomly selected locations revealed that *G. boliviana* was present at >70% of locations in south/central Florida (Overholt et al. 2009). Albeit highly anecdotal, of the two ranchers who provided sites for this study, one now depends entirely on biological control for TSA management, and the other only very infrequently uses small spot treatments with herbicide to augment biological control. We suspect that the use of chemical and mechanical methods of TSA control has greatly declined in central and south Florida since *G. boliviana* became widespread, and recommend that a second survey be conducted to determine current management practices and ascertain ranchers attitudes toward biological control and TSA management, in general.

One question of practical interest is whether additional biological control agents are needed to further reduce TSA density. Based on the information currently available, new agents are required for the northern peninsula and the panhandle, as a survey conducted in the fall of 2008 found no beetles or beetle damage north of 29° latitude (Overholt et al. 2009). In south/central Florida, the question is more difficult to answer. The present study showed that *G. boliviana* was able to reduce the density of TSA to around 1 plant/4 m^2. However, no economic injury level for TSA has been established, so it is difficult to determine whether the level of suppression provided by *G. boliviana* is sufficient. Moreover, our data show that outbreaks of the weed may still occur, as seen at Control site 2 in January, 2009. We believe that a biological control agent which continues to feed on TSA during winter months would compliment the activity of *G. boliviana* and may further suppress populations of TSA in central/south Florida.

Acknowledgements

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